HIGH PREVALENCE OF VITAMIN D DEFICIENCY IN 1048 ROMANIAN WOMEN WITH POSTMENOPAUSAL OSTEOPOROSIS#

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Abstract

Vitamin D deficiency and insufficiency are common medical problems worldwide as they are quite prevalent in both healthy adults and individuals with osteoporosis, hospitalized patients and free-living and institutionalized elderly. The lack of serum 25-hydroxy-vitamin D (25OHD) assays standardization, variability of reference population, and the use of different cut-off points have produced quite different prevalence reports from epidemiological studies.

We investigated the vitamin D status (deficiency, insufficiency, sufficiency) in 1048 Romanian postmenopausal women with osteoporosis referred to our clinic for diagnosis and treatment in the last three years. Most patients were untreated with osteoporosis drugs and non-supplemented with vitamin D. In our country dietary sources of vitamin D are scarce and there is no fortification of food with vitamin D. We found a high prevalence of both vitamin D deficiency (25OHD < 10 ng/mL) - 22.23% and insufficiency (25OHD=10-30 ng/mL) - 61.26%. Our study also revealed a high prevalence of low vitamin D when using other cut-offs as reported in the literature. 83.49% had values lower than 30 ng/mL and 60.97% lower than 20 ng/mL. In this study we identified a serum 25OHD concentration of 35 ng/mL above which serum parathyroid hormone (PTH) concentration attains a plateau at about 35 pg/mL. The relation between serum PTH and 25OHD concentration was non-linear and a log-log diagram showed a very weak correlation. The prevalence of secondary hyperparathyroidism was 32.25% in the whole population studied. It ranged from 40% in the subgroup of serum 25OHD less than 10 ng/mL to less than 15% in patients with 25OHD higher than 30 ng/mL.

In conclusion, in a representative osteoporosis population from Romania we found a very high prevalence of vitamin D deficiency and insufficiency whatever the cut-off used for definition.

Key words: osteoporosis, vitamin D deficiency, vitamin D insufficiency, secondary hyperparathyroidism.

Some of the data are included in "25-hydroxy-vitamin D and parathyroid hormone status in 834 postmenopausal women with osteoporosis" (Acta Endocrinologica – Buc, vol.I, no.3, p.1-7, 2005)

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INTRODUCTION

Vitamin D is essential for both calcium and bone homeostasis. Currently, the vitamin D status of an individual is assessed by measuring the serum concentrations of 25 hydroxy-vitamin D (25OHD). Classically, vitamin D deficiency, when prolonged and severe, can lead to osteomalacia, a condition characterized by insufficient mineralization of the osteoid. Vitamin D deficiency also causes muscle weakness and an increased risk of falls (1,2). Most clinicians agree that a serum 25OHD<10 ng/ml represents vitamin D deficiency (3), a cut-off that we also used in this paper. Other experts favour a cut-off less than 15 ng /ml and even less than 20 ng /ml, but they come from US populations, where the food is fortified with vitamin D and the supplementation is more frequent (21). The concept of vitamin D insufficiency has recently gained a remarkable interest as it was found quite prevalent both in normal population (adults and adolescents) (4-7), osteoporosis patients (8, 9) and community-dwelling elderly (10). This high prevalence makes vitamin D insufficiency a common health problem worldwide (9,11).

It has been proposed that inadequate concentrations of vitamin D lead to a calcium negative balance, secondary hyperparathyroidism, increased bone turnover, bone loss, and an increase in fracture risk (3).

A precise definition of vitamin D insufficiency requires a standard definition of optimal vitamin D status, which is actually not the case. A recent consensus panel, argued for optimal vitamin D serum concentrations clustered between 28-32 ng/mL (12), based on several criteria: the level associated with maximal suppression of serum PTH concentration (4,7,13), with the greatest calcium absorption (14), the highest bone mineral density (BMD) (15), reduced rates of falling (16), and reduced fracture rates (1,17). Most epidemiological studies that have assessed vitamin D status have been performed in Western Europe and the United States. Therefore, we undertook an analysis which aimed to determine the vitamin D status (deficiency, insufficiency, sufficiency) of postmenopausal osteoporosis patients and to estimate an optimal serum vitamin D concentration in Romanian patients based on the relation with serum PTH concentrations.

SUBJECTS AND METHODS

Study population

We investigated the vitamin D status in 1048 postmenopausal women with osteoporosis (diagnosed by DEXA using WHO criteria) referred to our clinic for diagnosis and treatment in the last 3 years. The study was conducted in the Osteoporosis Center of the National Institute of Endocrinology, Bucharest. Romania is geographically located in Central Europe, around 45^{o}N, with four seasons. Although we previously observed a seasonal variation in vitamin D concentration between summer and winter we did not adjust the values as they were

evenly distributed around the year. Most patients were untreated with osteoporosis drugs and non-supplemented with vitamin D. In Romania dietary sources of vitamin D are scarce and there is no fortification of food with vitamin D. The dietary vitamin D and calcium intakes were estimated by the investigators and were below the usual recommendations for postmenopausal women (200-300 mg calcium and less than 10 mcg vitamin D/day).

Our local protocol includes:

- past medical history, physical examination;
- diagnosis of secondary causes of osteoporosis by specific endocrine tests;
- dual X-ray absorptiometry (lumbar spine and hip)- for osteoporosis diagnosis;
- fractures evaluation (medical history for peripheral fracture, X-ray assessment for vertebral fractures);
- laboratory assessment: serum calcium, phosphorus, albumin, creatinine, alkaline phosphatase, and urinary calcium concentrations.
- in patients with low Z-score (<-1.5DS), multiple fractures, inadequate response to treatment, inadequate nutrition status we also include in the protocol the followings: serum 25OHD, intact PTH, bone turnover markers (serum cross-laps and osteocalcin).

Following the purpose of the study the patients with secondary causes of osteoporosis were excluded. We also carefully excluded primary hyperparathyroidism in high-PTH, vitamin D sufficient women (serum 25OHD>30 ng/ml) by reviewing the charts and the serum calcium (10-10.5 mg/dL) of our patients. In patients with high PTH and low 25OHD we first normalized vitamin D status and then categorized the patients. In this way the study group used for our analysis consisted of 1048 osteoporosis patients, with and without prevalent fractures, and with no other medical conditions related to bone and calcium metabolism. Postmenopausal status was defined by the menstrual history, hormonal data and by documented bilateral oophorectomy, in patients above 40 yrs old.

In a subgroup of 835 patients we also analyzed other characteristics besides BMD: BMI, urinary calcium, alkaline phosphatase.

Methods

BMD assessment

Lumbar spine and femoral BMD were assessed by dual-energy X-ray absorptiometry using GE Lunar Prodigy equipment. Vertebrae that could not be analyzed (osteophytes, fractures, artifacts) were excluded from the analysis. Long-term precision error of our equipment is less than 1% at the lumbar spine and less than 2% at the hip.

Laboratory assessment

A single blood sample was collected in the morning, in a fasting state, to assess 25OHD, intact PTH and the other laboratory measurements.

Serum 25OHD concentration was measured by ELISA (ImmunDiagnostic 25OHD EIA-kit) with an intrassay and interassay CV<10%, minimum detection limit of 1.4 ng/ml; normal range: 19-58 ng/mL.

Serum intact PTH was measured by ELISA (DSL-10-8000 ACTIVE^{TM} I-PTH, intrassay and interassay CV <6.3%, minimum detection limit of 1 pg/ml; reference values: 16-62 pg/mL. As the manufacturer did not specify if the individuals were vitamin D repleted we used 2 cut-offs for the upper level for the normal PTH: the one recommended by the manufacturer (62 ng/mL and another one determined by us in our patients from the relation between PTH-vitamin D serum concentrations (50 ng/mL).

Biochemical analyses were determined by automated standard laboratory methods.

Outcome measures

Vitamin D status

We categorized vitamin D levels in 3 subgroups, based on the most recent studies and an expert opinion (12,18,19):

- deficiency: <10 ng/mL
- insufficiency: 10-30 ng/mL
- sufficiency: > 30 ng/mL

As there is no common definition of optimal vitamin D status various cut-points of serum 25OHD were also used: <10 ng/mL, <15 ng/mL, <20 ng/mL, <25 ng/mL, <30 ng/mL, <35 ng/mL.

Relationship between PTH and 25OHD serum concentrations and the identification of vitamin D optimal level

Prevalence of secondary hyperparathyroidism

The percent of subjects with biochemical evidence of secondary hyperparathyroidism (as defined by normal serum calcium with a PTH value above the upper limit of the manufacturer's normal reference range and that resulted from our own analysis) was also determined.

Statistical analysis

ANOVA was used to assess differences in parameters across the vitamin D subgroups.

We performed a scatter plot of 25OH values *versus* PTH values which shows the lack of a linear correlation between these values. Although the correlation coefficient is -0.1995 and it is highly significant (t-Student test of equality with 0 gives t=8.22), the pattern of points on the scatter plot shows a rather nonlinear relation between the two parameters. However, it is extremely difficult to fit data with an exponential decay, this model being inappropriate. Rather a power decreasing function might be a better model but the fit, given by the model for $f(x)= \alpha/x$ for $x>0$ and some $\alpha>0$ to be determined, was very poor.

Also, we tried to use "the moving average" method, used on the purpose of obtaining an average curve approximating PTH as a function of 25OH. The result was influenced by the small number of cases with high values of 25OH and the obtained curve was very oscillating.

Due to the strong asymmetry of both distributions, we have performed a log transformation of all data and the correlation coefficient on the transformed data

was r=-0.159, again highly significant (t-Student =5.76), but the regression of PTH against 25OH shows a pattern of residual plots which suggests a nonlinear relation again.

A surrogate of a cut-off value can be obtained by dividing the range of 25OH values in small intervals and trying to test the differences between average values of PTH in the neighbouring intervals. If each interval contains enough 25OH values, the t-test has both significance and power. We have set the significance level at 5% and the power of every test at 80%. The intervals and the result we have obtained are presented below.

The statistical analysis of the relationship between 25OHD and PTH was performed by Professor Petre Badea, Department of Medical Biostatistics, University of Medicine and Pharmacy, Craiova.

RESULTS

Comparison of vitamin D subgroups

From our group of 1048 patients, the characteristics of 835 patients (mean± SD) grouped according to their vitamin D status are presented in Table 1. The women in the three vitamin D subgroups were similar in prevalence of femoral neck BMD (data not shown). However, the women who were vitamin D deficient were slightly older (57.2 ±12.5 compared with 55.2 ±11.6 yr) than those with vitamin D sufficiency. The sufficient group had a higher body mass index (BMI) when compared with the deficient group (25.1 ± 4.0 *vs*. 24.2 ± 4.3 kg/m^2) but not with the insufficient group (24.9 ± 3.7 kg/m^2). The women with vitamin D insufficiency had higher BMD at the spine than those with deficiency and insufficiency (0.81 ± 0.14 g/cm^2 compared with 0.78 ± 0.15 and 0.78 ± 0.13 g/cm^2, respectively). Serum calcium levels were similar among the three vitamin D status groups (data not shown). However, the vitamin D-deficient category had the highest serum PTH level compared with the insufficient and sufficient groups (45.97 ± 25.09 *vs*. 38.70 ± 20.37 and 35.15 ± 16.55 pg/mL, respectively).

The deficient patients had higher serum alkaline phosphatase levels compared with the sufficient and insufficient patients (101.96 ± 72.95 *vs.* 73.99 ± 39.61 and 85.04 ± 50.93 U/L, respectively). Also, the deficient patients had lower urinary calcium compared with sufficient and insufficient patients (0.15 ± 0.09 *vs*. 0.21 ± 0.13 and 0.17 ± 0.08 g/24h, respectively) - Table 1.

Distribution of serum 25OHD

The mean (SD) serum 25OHD was 20.2 ng/mL (14.8) and the distribution was asymmetric (with a maximum value of 100 ng/mL). The median value was 16.76 ng/mL. The discrepancy between the mean and median was due to the high numbers of values in the deficient and insufficient range. We also had lower numbers in the sufficient category and we carefully excluded patients with normocalcemic primary hyperparathyroidism (patients with high PTH and 25OH serum levels) (Fig. 1).

Table 1. Characteristics of study participants grouped according to their vitamin D status

	Vitamin D deficiency 25OHD<=10 ng/mL	Vitamin D insufficiency 10<25OHD<=30 ng/mL	Vitamin D sufficiency 25OHD>30 ng/mL
n	214	466	155
25OHD [ng/mL]	6.70±2.29	18.15±5.39	44.13±12.57
Age [years]	57.21±12.56	55.88±5.39	55.27±11.66
BMI [Kg/m2]	24.21±4.37	24.95±3.74	25.19±4.09
BMD [g/cm2]	0.78±0.15	0.81±0.14	0.78±0.13
PTH [pg/mL]	45.97±25.09	38.70±20.37 [a]	35.15±16.55 [a,b]
Urinary Ca[g/24h]	0.15±0.09	0.17±0.08 [c]	0.21±0.13 [d]
AlkPh[U/L]	101.96±72.95	85.04±50.93 [e]	73.99±39.61 [a,f]

[a] p<<0.001 compared with vitamin D deficient
[b] p=0.02 compared with vitamin D insufficient
[c] p=0.07 compared with vitamin D deficient
[d] p<0.01 compared with vitamin D deficient and insufficient
[e] p=0.03 compared with vitamin D deficient
[f] p=0.09 compared with vitamin D insufficient

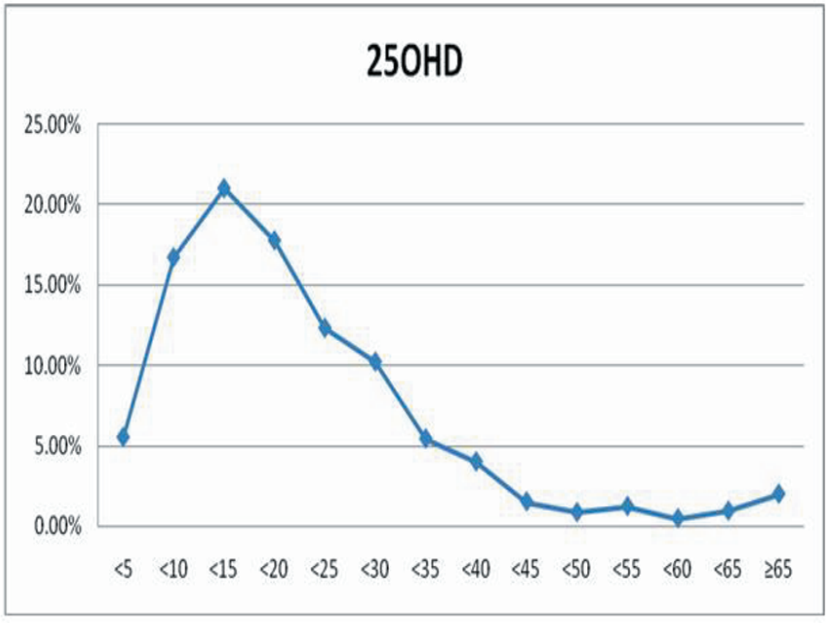

Figure 1. Distribution of serum 25OHD concentrations (ng/mL).

Table 2. Prevalence of vitamin D deficiency, insufficiency and sufficiency

	10	10-30	=30
N=1048	N=233	N=642	N=173
Prevalence	22.23%	61.26%	16.51%
Age [years]	59.68±10.19	57.42±10.79	55.64±10.68
25OHD [ng/mL]	6.73±2.37	18.32±5.47	45.53±17.20
PTH [pg/mL]	52.37±28.85	47.42±24.98[a]	35.33±14.23[b,c]

[a] p<0.001 compared with vitamin D deficient
[b] p<<0.001 compared with vitamin D deficient
[c] p<<0.001 compared with vitamin D insufficient

Prevalence of low serum 25OH concentration

Twenty two percent of patients were vitamin D deficient (serum 25OHD less than 10 ng/mL). The mean (SD) serum 25OHD was 6.37 ng/mL (2.37). These patients were older and had higher mean serum PTH (52.37 ± 28.85 pg/mL) than the sufficient and insufficient groups (p<0.001). 61.26% of patients were vitamin D insufficient (serum 25OHD between 10-30 ng/mL). The mean (SD) serum 25OHD was 18.32 ng/mL (5.47). These patients were slightly older and had higher mean serum PTH (47.42 ± 24.98 pg/mL) than the sufficient group (35.33 ± 14.23 pg/mL) (p<0.001). 16.51% of patients were vitamin D sufficient (serum 25OHD higher than 30 ng/mL). The mean (SD) serum 25OHD was 35.33 ng/mL (14.23) (Table 2).

Several other cut points were used to describe the prevalence of vitamin D inadequacy in the study population: 60.97% of patients had a serum 25OHD less than 20 ng/mL and 83.49% had values less than 30 ng/mL (Fig. 2).

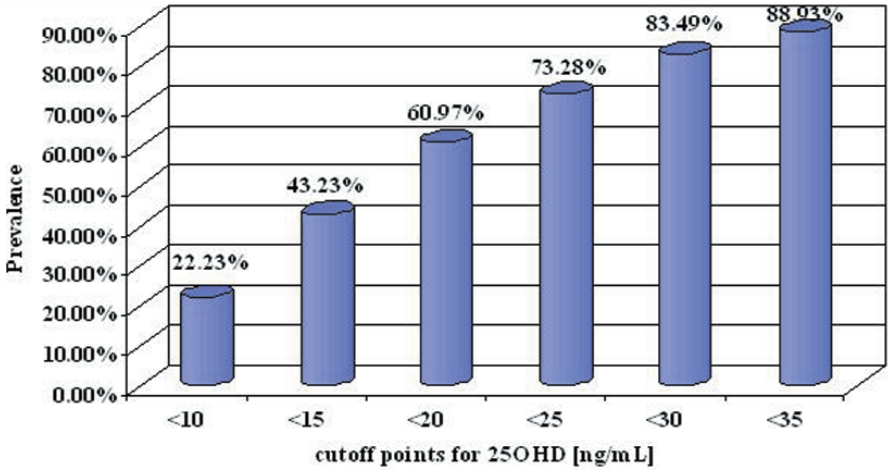

Figure 2. Prevalence of vitamin D inadequacy with different cut-point values (ng/mL).

Relationship between serum PTH and 25OHD concentrations

Looking at the serum 25OHD and PTH concentrations for all 1048 patients we realized that the relation is non-linear. Therefore, we used the diagram of log PTH concentration *vs*. log 25OHD concentration and we observed a very weak correlation ($r^2 = 0.0255$). That is why it was not possible to find a best fit curve for our data, despite the use of several approaches, like moving average and smooth fitting curve. To compare in a different way the effects of 25OHD concentration on PTH, the patients were grouped according to their 25OHD concentration, in 5 ng/ml increments. Then we compared the mean (SD) serum PTH (pg/mL) between pairs of adjacent vitamin D subgroups (Fig. 3). In this way we obtained a significant difference (p=0.04) in serum PTH concentration at a serum 25OHD of 35 ng/mL. Beyond this 25OHD concentration, serum PTH plateaus at about 35 pg/mL.

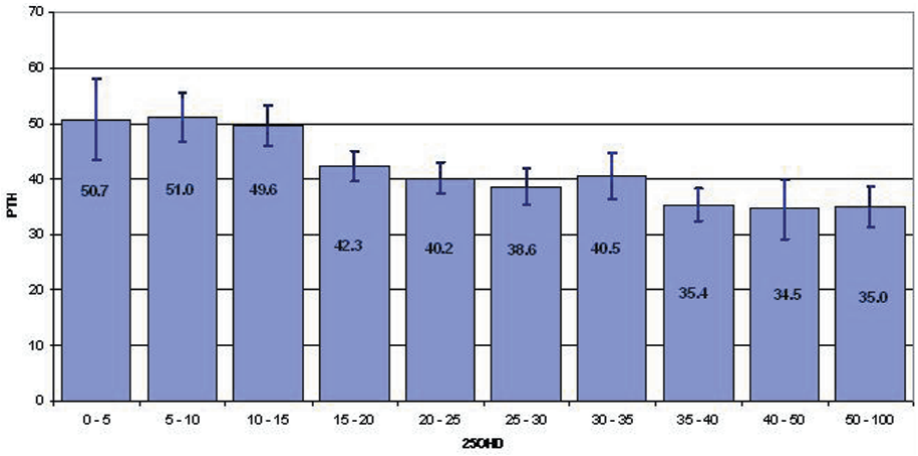

Figure 3. Mean (±SD) serum PTH (pg/mL) by serum 25OHD subgroups (ng/mL).

Prevalence of secondary hyperparathyroidism

In this study we used two cut-points to define the upper normal range of serum PTH - the one recommended by the manufacturer at 62 pg/mL and the other one calculated by us (50 pg/mL). In the overall study population of 1048 patients the prevalence of secondary hyperparathyroidism was 19.47% for the first cut-point and 32.25% for the second one. Looking at the subgroups of serum 25OHD concentrations, the prevalence of secondary hyperparathyroidism was between 24.89% - 39.91% in the deficient group and decreased to less than 7.02-15.79% at a serum concentration higher than 30 ng/mL (Fig. 4).

DISCUSSION

In this study of 1048 women with postmenopausal osteoporosis who live in Romania we found a high prevalence of both vitamin D deficiency (22.23%) and

insufficiency (61.26%). Although similar findings regarding the prevalence of insufficiency have been reported in previous studies, our study was the first to report such a high prevalence of vitamin D deficiency in a representative osteoporosis population living at 45ºN, in Central Europe. The prevalence of vitamin D deficiency observed in this study (22.23%) is much higher than that reported in other osteoporosis population using the same cut-point (less than 10 ng/mL): 4% (9), 4.4% (19), 1.1% (8). A prevalence between 12.5-22.6% has been reported in other countries from Central Europe but on a small number of patients (9). Comparing with other studies it is obviously inadequate as other cut-points were used for deficiency (less than 15 and less than 20 ng/mL) (11). The sample size seems also to influence the reported prevalence.

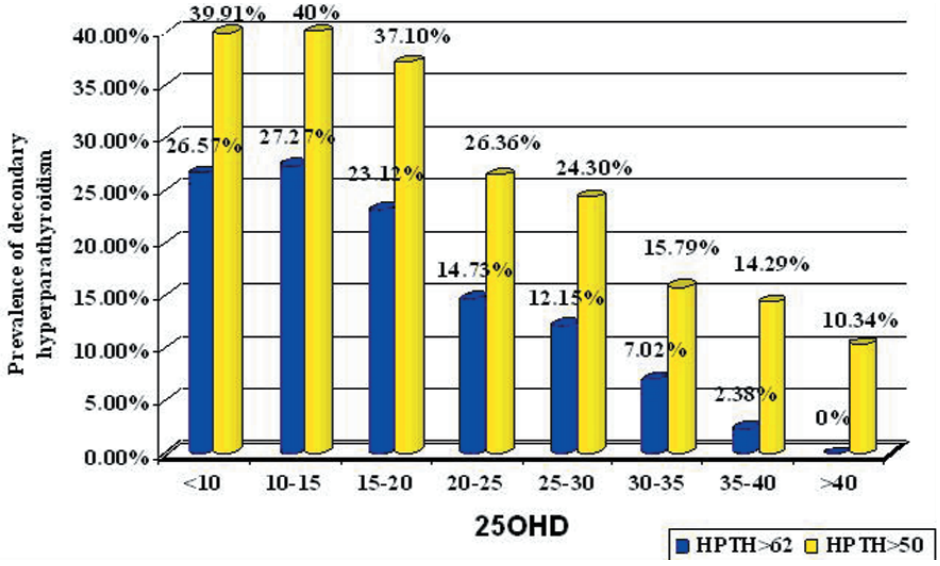

Figure 4. Percent of subjects with secondary hyperparathyroidism in vitamin D subgroups (ng/mL).

As such in three studies of osteoporosis patients and with a cut-point of less than 12 ng/mL the prevalence was between 27-76% at a sample size between 62-700 patients (11).

Paradoxically, it is the reported a high prevalence of vitamin D deficiency in healthy adults -26.2% (less than 10 ng/mL) in Finland (5) and 14 % in France (less than 12 ng/mL) (4) and 36% in independently elderly in France (20). Vitamin D deficiency is also very common in patients with hip fracture, hospitalized patients and institutionalized elderly (3).

Our study also revealed a high prevalence of low vitamin D when using other cut-offs as reported in the literature. Therefore, 83.49% had values lower than 30 ng/mL and 60.97% lower than 20 ng/mL. As in other European countries, in Romania dietary sources of vitamin D are scarce, there is no food fortification and no consistent supplementation with calcium and vitamin D. This remarkable high prevalence has

important implications for clinical practice, almost every woman with osteoporosis in Romania can be *a priori* considered to have a certain degree of vitamin D inadequacy. It is common practice in our osteoporosis center to recommend supplementation with calcium and vitamin D to every patient with osteoporosis. At the same time these data do not support a vitamin D screening in osteoporosis patients. On the other side, our data support the use, in clinical practice, of urinary calcium and serum alkaline phosphatase to help identify vitamin D deficient patients when 25OHD is not available.

Vitamin D insufficiency defined as serum 25OHD between 10-30 ng/mL was also very prevalent in this study - 61.26% - confirming it as a common medical problem worldwide.

There are a lot of controversies regarding the definition of different categories of low vitamin D and of the precise estimation of optimal vitamin D status. Usually, in Europe, a threshold of 20 ng/mL is agreed (3), although in the USA a 30 ng/mL is favoured (21). There are several problems that need to be clarified before establishing these cut-off values; they were elegantly reviewed recently (3,21,22).

We agree that using population-based references could be misleading as we think that the large variability of data is also derived from differences in the population evaluated: geographical location, dietary and clothing habits, age, race, etc. As such using as a reference the range of vitamin D levels in normal premenopausal women we found quite a different prevalence of deficiency and insufficiency from that presented in this study (data not shown). The other important sources of variability are the lack of standardization of vitamin D assays and the large variability of our measurements.

This and the low number of patients with sufficient levels (>30 ng/mL) precluded us, maybe, to find a best fit curve for the relation between PTH and 25OHD concentrations in this study. This kind of arguments made experts recommend searching for optimal vitamin D status using serum 25OHD values which prevent an adverse health outcome (3). Some of these biomarkers, reflecting the tissular action of vitamin D for calcemic and non-calcemic effects, were then characterized (15). Some of them are reminiscent of calcium homeostasis, a main action of vitamin D, but others are closer to pharmacology (optimal concentration to prevent fracture and falls). For the endocrinologist thinking of searching for different optimal concentrations for different actions is confusing. Vitamin D could be considered a nutrient with threshold behaviour but actually it is a special hormone which needs activation in our body in order to be fully active on its numerous calcemic and non-calcemic actions. This is reminiscent of thyroid and androgen physiology, when a circulating precursor is activated in a target organ. As such vitamin D could also be activated in bone tissue as in many other tissues. Therefore, it would be more rewarding to search for optimal tissue concentration than for serum concentration.

Several criteria have been proposed for defining the optimal vitamin D status: the level associated with maximal suppression of serum PTH concentration (4,7,13), with the greatest calcium absorption (14), the highest bone mineral density (BMD) (15), reduced rates of falling (16), and reduced fracture rates (1,17).

Numerous studies were in the search for the threshold of secondary

hyperparathyroidism. Estimates of the 25 OHD level needed for maximum suppression of PTH have been placed at 12 ng/mL (23), 20 ng/mL (7), 26.30 ng/mL (24), 28-30 ng/mL (25), 29.8 ng/mL (8), 30-32 ng/mL (4), 39.6 ng/mL (26).

In this study we identified a serum vitamin D concentration of 35 ng/mL above which serum PTH concentration attains a plateau at about 35 pg/mL, which is close to the cluster of values identified by an expert panel at 30-32 ng/mL (12).

There is some disagreement as to whether PTH truly attains a lower plateau as serum 25OHD level increases (13). We think this is the consequence of sparse data for 25OHD in the high level range common in many studies.

Secondary hyperparathyroidism has been proposed as the principal mechanism whereby low vitamin D status could contribute to the increased risk of fracture and especially to the pathogenesis of hip fractures. As mentioned, in this study we used two cut-points to define the upper normal range of serum PTH - the one recommended by the manufacturer at 62 pg/mL and the other one calculated by us (50 pg/mL). Using the last cut-point we found the prevalence of secondary hyperparathyroidism to be higher than using the former one, and a decrease from 40% at lower serum 25OHD concentrations to less than 15% at serum levels higher than 30 ng/mL.

Serum PTH correlated negatively with serum 25OHD in many studies (4, 8,13,27,28), usually with a correlation coefficient between 0.20-0.30, which is a weak correlation. This correlation has been estimated with a different approach in different studies. For some of them the best fit was obtained by a linear regression model while others obtained it by a non-linear regression analysis. Our own data showed that the relation is non-linear, and in the log-log diagram we found a significant but very weak correlation (r=-0.159). In other words, our data did not allow us to find the best curve to fit these values. Using another approach we were able to identify a clear plateau of serum PTH at about 35 pg/mL for serum 25OHD above 35 ng/mL.

As the prevalence of secondary hyperparathyroidism in patients with 25OHD deficiency is only 40% in our study, implies that biochemical evidence of secondary hyperparathyroidism may not be the most sensitive indicator of vitamin D inadequacy in an individual patient.

In conclusion, in a representative osteoporosis population of Romania we found a very high prevalence of vitamin D deficiency and insufficiency whatever the cut-off used for definition. We hope that these data will serve as a basis for a coherent policy of public health in Romania.

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